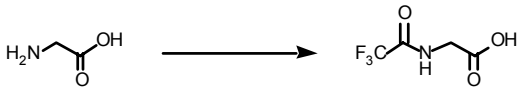
Supporting information for Chen *et al.* (2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.182542899

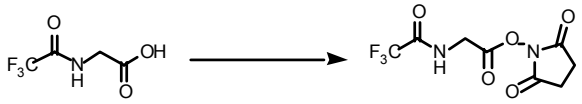
Supporting Text

General Synthetic Procedures. All reactions were performed under a positive pressure of nitrogen. Air- and moisture-sensitive compounds were introduced via syringe or cannula through a rubber septum. All solvents were analytical grade and used as received from commercial sources, and flash chromatography purifications were performed with the indicated solvent system on E. Merck silica gel 60 (230-400 mesh). Structure confirmation of the synthetic compounds was achieved through proton NMR and mass spectrometry.



1

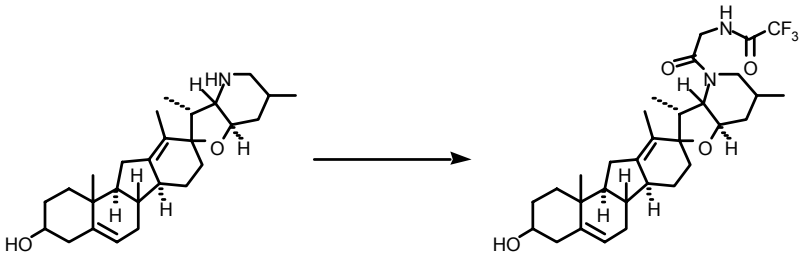
***N*-Trifluoroacetyl Glycine (1).** Methyl trifluoroacetate (804 μl, 7.99 mmol) and triethylamine (928 μl, 6.66 mmol) were added to a suspension of glycine (500 mg, 6.66 mmol) in methanol (2.5 ml). After the mixture was stirred vigorously for 18 h, 1 M HCl was added dropwise until a pH of 2 was obtained. The reaction was added to ethyl acetate (30 ml), washed with 1 M HCl (2 × 10 ml), dried over $MgSO_4$, and concentrated *in vacuo* to yield the amide as a white solid (991 mg, 5.79 mmol, 87%).



1 **2**

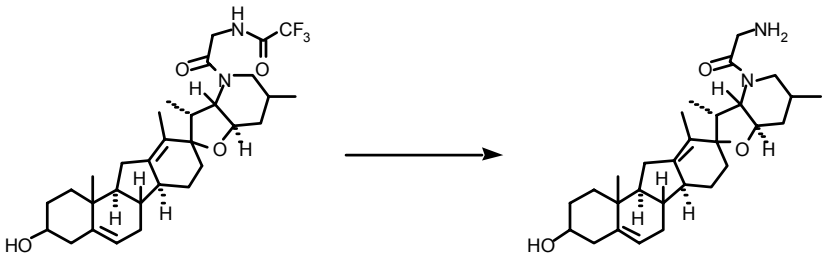
***N*-Trifluoroacetyl Glycine *N*-Hydrosuccinimide Ester (2).** Disuccinimidyl carbonate (300 mg, 1.17 mmol) was added to a solution of **1** (200 mg, 1.17 mmol) and pyridine (94.6 μl, 1.17 mmol) in acetonitrile (1.0 ml). The reaction was stirred at room

temperature for 3 h, during which the solution became clear and evolved gas. The reaction mixture was added to ethyl acetate (10 ml), washed with 1 M HCl (2 × 5 ml) and saturated aqueous $NaHCO_3$ (2 × 5 ml), dried over $MgSO_4$, and concentrated *in vacuo* to yield a white solid (232 mg, 865 µmol, 74%).



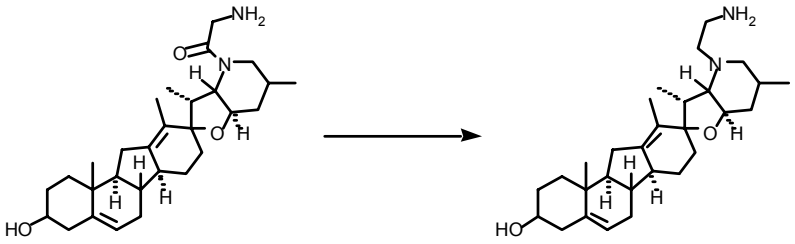
3

***N*-(*N*'-Trifluoroacetyl Glycyl) Cyclopamine (3).** Triethylamine (135 µl, 972 µmol) and **2** (261 mg, 972 µmol) were added to a solution of cyclopamine (200 mg, 487 µmol) in dichloromethane (2.0 ml). The reaction was stirred at room temperature for 1 h and then subjected directly to purification by flash chromatography (SiO_2, step-wise gradient from 8:1 to 2:1 hexane/acetone) yielded the amide as a white solid (166 mg, 294 µmol, 60%).



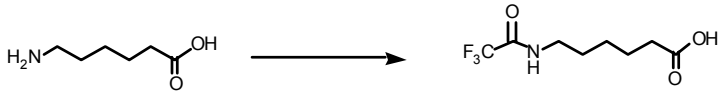
3 **4**

***N*-Glycyl Cyclopamine (4).** Aqueous ammonia [3 ml of a 29% (w/w) solution in water, 45.6 mmol] was added to a solution of **3** (162 mg, 296 µmol) in methanol (4 ml). The reaction was stirred at room temperature for 5 h and then evaporated to dryness *in vacuo*. Purification by flash chromatography (SiO_2; chloroform, then step-wise gradient from 20:1:0.1 to 20:2:0.1 chloroform/methanol/triethylamine) yielded the amine as a white solid (110 mg, 235 µmol, 79%).



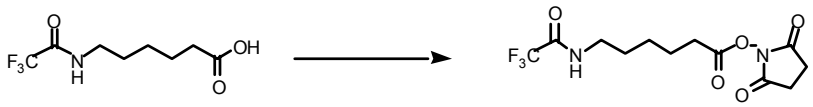
4 **5**

***N*-Aminoethyl Cyclopamine (5).** Lithium aluminum hydride (939 µl of a 1 M solution in THF, 939 µmol) was added to a suspension of **4** (110 mg, 235 µmol) in THF (6 ml). The reaction was refluxed for 3 h and then quenched with water (5 ml) and aqueous KOH (10 ml of a 10% solution). After extracting the mixture with chloroform (2 × 20 ml), the organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, step-wise gradient from 20:1:0.1 to 20:2:0.1 chloroform/methanol/triethylamine) yielded the diamine as a colorless oil (94.4 mg, 208 µmol, 88%).



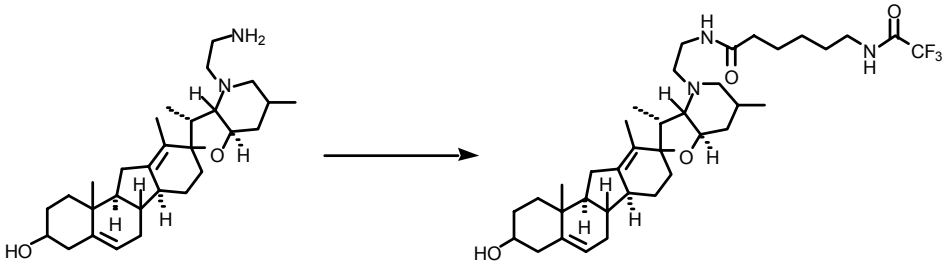
6

***N*-Trifluoroacetyl Aminocaproic Acid (6).** Methyl trifluoroacetate (513 µl, 5.10 mmol) and triethylamine (474 µl, 3.40 mmol) were added to a suspension of aminocaproic acid (455 mg, 3.40 mmol) in methanol (2 ml). After the mixture was stirred vigorously for 8 h, 1 M HCl was added dropwise until a pH of 2 was obtained. The reaction was added to ethyl acetate (10 ml), washed with 1 M HCl (2 × 2 ml), dried over $MgSO_4$, and concentrated *in vacuo* to yield the amide as a white solid (745 mg, 3.49 mmol, 100%.).



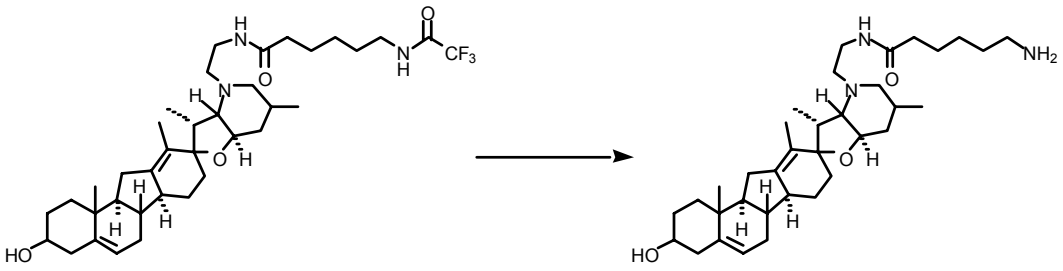
6 **7**

***N*-Trifluoroacetyl Aminocaproic Acid *N*-Hydroxysuccinimide Ester (7).**
Disuccinimidyl carbonate (541 mg, 2.11 mmol) was added to a solution of **6** (300 mg, 1.41 mmol) and pyridine (227 µl, 2.81 mmol) in acetonitrile (2.0 ml). The reaction mixture was stirred at room temperature for 13 h, during which the solution became clear and evolved gas. The solution was added to ethyl acetate (10 ml), washed with 1 M HCl (2 × 1 ml) and saturated aqueous $NaHCO_3$ (2 × 1ml), dried over $MgSO_4$, and concentrated *in vacuo* to yield a white solid (471 mg, 1.45 mmol, 100%.).



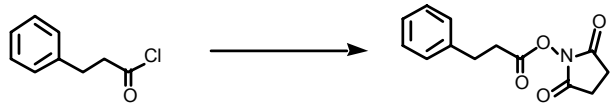
5 **8**

***N*-(*N*'-(*N*''-Trifluoroacetyl Aminocaproyl) Aminoethyl) Cyclopamine (8).**
Triethylamine (12.3 µl, 88.0 µmol) and **7** (17.1 mg, 52.8 µmol) were added to a solution of **5** (20.0 mg, 44.0 µmol) in dichloromethane (200 µl). The reaction mixture was stirred at room temperature for 13 h, quenched with dimethylaminopropylamine (11.2 µl, 88.0 µmol), and evaporated to dryness with a stream of nitrogen gas. Purification by flash chromatography (SiO_2, step-wise gradient from 50:1 to 25:1 chloroform/methanol) yielded the amide as a colorless oil (26.9 mg, 40.5 µmol, 92%).



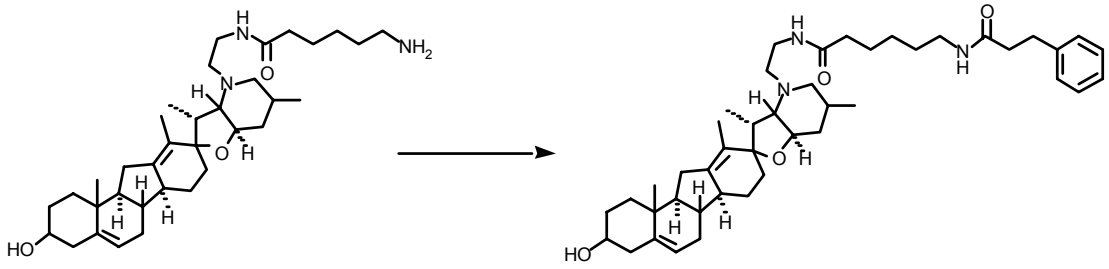
8 **9**

***N*-(*N'*-Aminocaproyl Aminoethyl) Cyclopamine (9).** Aqueous ammonia [200 µl of a 29% (w/w) solution in water, 3.04 mmol] was added to a solution of **8** (26.9 mg, 40.5 µmol) in methanol (400 µl). The reaction was stirred at room temperature for 20 h and then evaporated to dryness by a stream of nitrogen gas. Purification by flash chromatography (SiO_2, step-wise gradient from 20:1:0.1 to 20:4:0.1 chloroform/methanol/triethylamine) yielded the amine as a white waxy solid (19.3 mg, 34.0 µmol, 84%.).



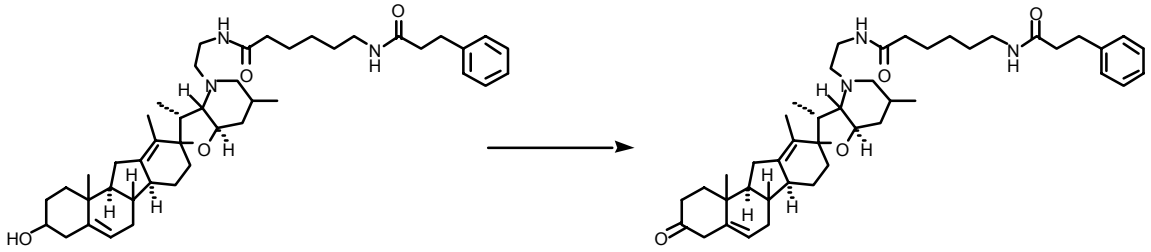
10

Dihydrocinnamic Acid *N*-Hydroxysuccinimide Ester (10). Dihydrocinnnamoyl chloride (638 µl, 4.21 mmol) was added dropwise to a solution of *N*-hydroxysuccinimide (500 mg, 4.21 mmol) and triethylamine (704 µl, 5.05 mmol) in dichloromethane (5 ml) at 0°C. The reaction was warmed to room temperature and stirred for 1 h. The reaction mixture was then added to diethyl ether (50 ml), washed with 1 M HCl (1 × 20 ml) and saturated aqueous $NaHCO_3$ (1 × 20 ml), dried over $MgSO_4$, and concentrated *in vacuo* to yield a white solid (1.03 g, 4.17 mmol, 99%).



9 **11**

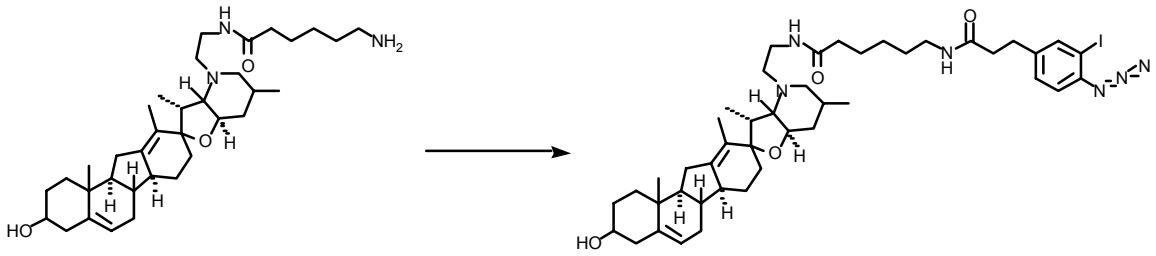
***N*-(*N*'-(*N*''-Dihydrocinnamoyl Aminocaproyl) Aminoethyl) Cyclopamine (11).** Triethylamine (88.1 µl, 63.2 µmol) and **10** (16.0 mg, 63.2 µmol) were added to a solution of **9** (18.0 mg, 31.6 µmol) in dichloromethane (1.5 ml). The reaction was stirred at room temperature for 1 h and evaporated to dryness by a stream of nitrogen gas. Purification by flash chromatography (SiO_2, step-wise gradient from 100:1 to 25:1 chloroform/methanol) yielded the amide as a colorless oil (11.5 mg, 16.4 µmol, 52%).



11 **12**

3-Keto, *N*-(*N*'-(*N*''-Dihydrocinnamoyl Aminocaproyl) Aminoethyl) Cyclopamine (KAAD-Cyclopamine; 12). Dimethylsulfoxide (12.7 µl, 177 µmol) was added to a solution of oxalyl chloride (7.73 µl, 88.6 µmol) in dichloromethane (250 µl) at –78°C. After the mixture was stirred at –78°C for 10 min, a solution of **11** (6.2 mg, 8.86 µmol) in dichloromethane (250 µl) was added, and the reaction was stirred at –78°C for another 30

min. The oxidation was completed by the addition of triethylamine (37.1 µl, 266 µmol) to the solution, which was stirred at –78°C for 10 min and then allowed to warm to room temperature. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (2 ml) and extracted with chloroform (2 × 2 ml). The resultant organic layer was then isolated, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, step-wise gradient from 100:1 to 25:1 chloroform/methanol) yielded the ketone as a slightly yellow oil (5.4 mg, 7.74 µmol, 87%).

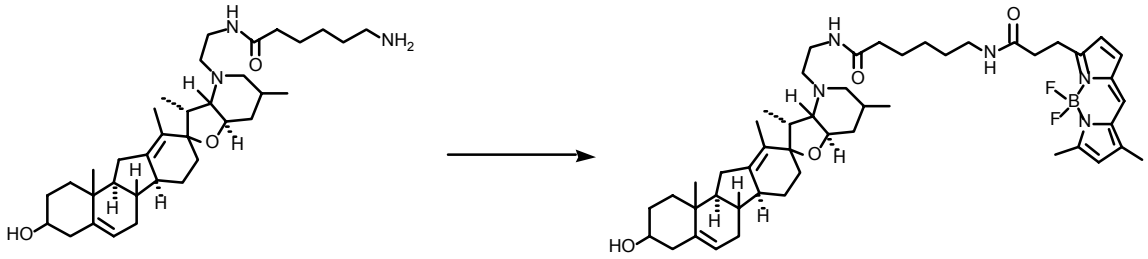


9 **13**

***N*-(*N*'-(*N*''-Azidoiodophenylpropionyl Aminocaproyl) Aminoethyl) Cyclopamine (PA-cyclopamine; 13)**. Azidoiodophenylpropionyl *N*-hydroxysuccinimide ester (1.4 mg, 3.38 µmol) and triethylamine (0.94 µl, 6.76 µmol) were added to a solution of **9** (1.92 mg, 3.38 µmol) in dichloromethane (250 µl). The reaction was stirred at room temperature for 2.5 h and evaporated to dryness by a stream of nitrogen gas. Purification by flash chromatography (SiO_2, step-wise gradient from 4:1:0.025 to 1:2:0.015 hexane/acetone/triethylamine) yielded the azide as a colorless oil (2.2 mg, 2.54 µmol, 75%).

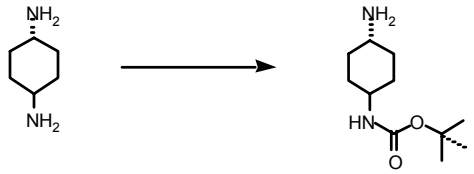
Preparation of ^{125}I-Labeled 13. ^{125}I-labeled azidoiodophenylpropionyl *N*-hydroxysuccinimide ester [0.250 mCi (1 Ci = 37 GBq), specific activity = 2200 Ci/mmol, 114 pmol; Marty Arbabian and Dr. Arnold Ruoho, Univ. of Wisconsin] in ethyl acetate (~1 ml) was concentrated to a volume of approximately 10 µl by a stream of nitrogen gas. The concentrated solution was diluted with ethyl acetate (100 µl) and was mixed with **9** (1.0 mg, 1.76 µmol) in chloroform (100 µl). The reaction mixture was incubated without stirring for 43 h at room temperature and then concentrated to approximately 10 µl by a

stream of nitrogen gas. The residue was resuspended in chloroform (200 µl) and purified by flash chromatography (SiO_2, step-wise gradient from 100:1 to 12.5:1 chloroform/methanol) to yield the radiolabeled azide. Fractions containing the desired product were pooled, concentrated by a stream of nitrogen gas and resuspended in methanol (250 µl). The solution was then reconcentrated by a stream of nitrogen gas, resuspended in methanol (250 µl), and stored at –20°C in the dark.



9 **14**

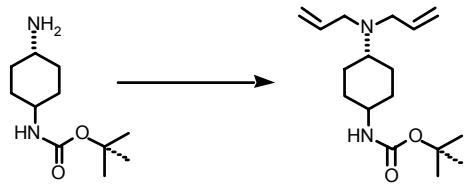
***N*-(*N*'-(*N*"-BODIPY FL Aminocaproyl) Aminoethyl) Cyclopamine (BODIPY-Cyclopamine; 14)**. BODIPY FL *N*-hydroxysuccinimide ester (2.0 mg, 5.28 µmol) and triethylamine (0.98 µl, 7.04 µmol) were added to a solution of **9** (2.0 mg, 3.52 µmol) in dichloromethane (500 µl). The reaction was stirred at room temperature for 20 h and then evaporated to dryness with a stream of nitrogen gas. Purification by flash chromatography (SiO_2, step-wise gradient from 50:1 to 12.5:1 chloroform/methanol) yielded the desired compound as a fluorescent green oil (2.6 mg, 3.09 µmol, 88%).



15

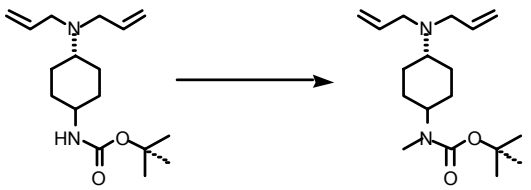
***N*-Boc-1,4-Diaminocyclohexane (15).** BOC-ON (1.24 g, 5.05 mmol) and triethylamine (1.41 ml, 10.1 mmol) were added to a solution of 1,4-diaminocyclohexane (577 mg, 5.05 mmol) in dichloromethane (10 ml). The reaction was stirred at room temperature for 2 h

and then evaporated to dryness *in vacuo*. Purification by flash chromatography (SiO_2, step-wise gradient from 40:1:0 to 10:1:0.1 chloroform/methanol/triethylamine) yielded the mono-protected compound as a white solid (521 mg, 2.43 mmol, 48%).



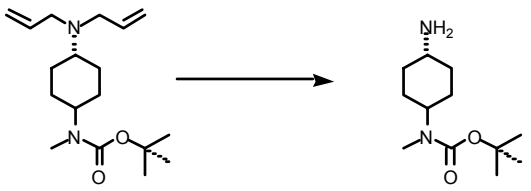
15 **16**

***N*-Boc-*N'*,*N'*-Diallyl-1,4-Diaminocyclohexane (16).** Allyl bromide (789 µl, 9.33 mmol) and diisopropylethylamine (447 µl, 2.61 mmol) were added to a solution of **15** (200 mg, 933 µmol) in DMF (4 ml). The reaction was stirred at room temperature for 18 h, and solvents were then removed *in vacuo* at 40°C. The resulting residue was dissolved in chloroform (6 ml) and washed with saturated aqueous $NaHCO_3$ (2 × 3 ml). The organic layer was then isolated, dried over $MgSO_4$, and concentrated *in vacuo* to yield the tertiary amine as a slightly yellow solid (113 mg, 384 µmol, 41%).



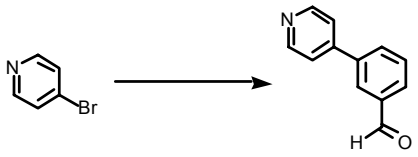
16 **17**

***N*-Boc-*N*-Methyl-*N*',*N*'-Diallyl-1,4-Diaminocyclohexane (17).** Lithium aluminum hydride (881 µl of a 1 M solution in THF, 881 µmol) was added to a solution of **16** (51.9 mg, 176 µmol) in THF (2 ml). The reaction was refluxed for 1 h and then quenched with water (5 ml) and aqueous KOH (10 ml of a 10% solution). After extracting the mixture with chloroform (3 × 10 ml), the organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The resultant residue was then dissolved in dichloromethane (2 ml) and treated with triethylamine (49.1 µl, 352 µmol) and Boc_2O (57.6 mg, 264 µmol). This second reaction was stirred at room temperature for 2 h. Purification by flash chromatography (SiO_2, step-wise gradient from 50:1 to 12.5:1 chloroform/methanol) yielded the methylated carbamate as a colorless oil (49.0 mg, 159 µmol, 90%).



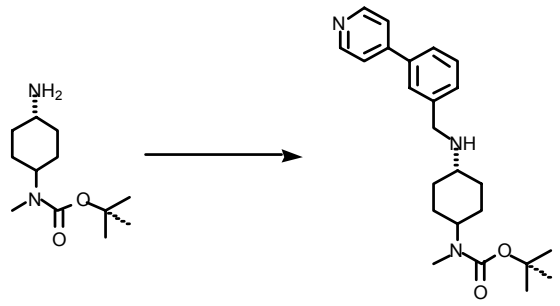
17 **18**

***N*-Boc-*N*-Methyl-1,4-Diaminocyclohexane (18).** $(Ph_3P)_3RhCl$ (6.00 mg, 6.54 μmol) was added to a solution of **17** (10.1 mg, 32.7 μmol) in acetonitrile:water (2 ml; 84:16 ratio). The mixture was then vigorously refluxed using a short path distillation apparatus, and fresh acetonitrile:water (5 ml over a period of 30 min; 84:16 ratio) was periodically added to the reaction to replaced distilled solvent. After refluxing for 30 min, the remaining solvent was removed *in vacuo*. Purification of resultant residue by flash chromatography (SiO_2, step-wise gradient from 20:1:0.2 to 5:1:0.05 chloroform/methanol/triethylamine) yielded the amine as a yellow solid (4.64 mg, 20.3 μmol, 62%).



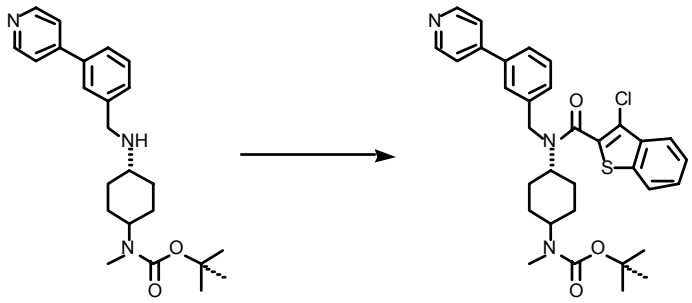
19

3-Pyridinylbenzaldehyde (19). A slurry of 4-bromopyridine (250 mg, 1.29 mmol) in a mixture of water (2 ml) and toluene (2.8 ml) was cooled to 0°C and treated a solution of Na_2CO_3 (314 mg, 2.97 mmol) in water (3.2 ml). After the mixture was allowed to warm to room temperature, 3-formylphenylboronic acid (202 mg, 1.35 mmol) and $Pd(Ph_3P)_4$ (74.5 mg, 64.5 μmol) was added. The reaction was then stirred at 85°C for 18 h. The resultant mixture was then added to dichloromethane (5 ml), and the organic and aqueous layers were separated. The aqueous layer was extracted further with dichloromethane (2 × 2 ml) and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, step-wise gradient from 4:1 to 1:4 hexane/ethyl acetate) yielded the biaryl compound as a colorless oil (204 mg, 1.11 mmol, 86%).



18 **20**

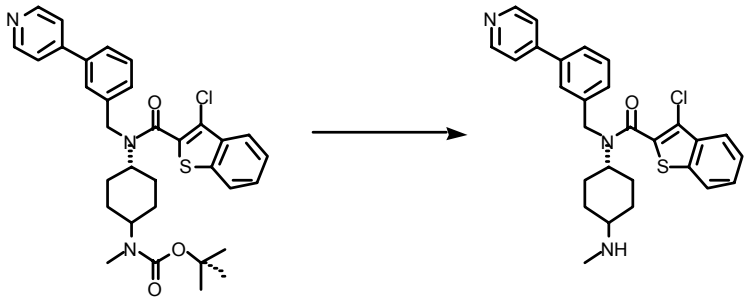
***N*-Boc-*N*-Methyl-*N*'-(3-Pyridinylbenzyl)-1,4-Diaminocyclohexane (20).** A solution of **18** (19.7 mg, 86.3 μmol) and **19** (15.8 mg, 86.3 μmol) in ethanol (2 ml) was treated with 4 Å molecular sieves and stirred at 70°C for 5 h. The mixture was allowed to cool to room temperature, upon which $NaBH_4$ (21.5 mg, 558 μmol) was added. After the reaction was stirred at room temperature for 12 h, it was treated with saturated aqueous $NaHCO_3$ (2 ml) and the aqueous layer was extracted with chloroform (3 × 3 ml). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, step-wise gradient from 20:1 to 5:1 dichloromethane/ethanol) yielded the secondary amine as a white solid (16.4 mg, 41.5 μmol, 48%).



20 **21**

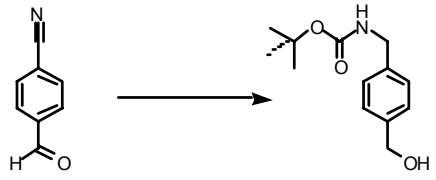
***N*-Boc-*N*-Methyl-*N*'-(3-Pyridinylbenzyl)-*N*'-(3-Chlorobenzo[b]thiophene-2-carbonyl)-1,4-Diaminocyclohexane (21).** 3-Chlorobenzo[b]thiophene-2-carboxylic acid (3.97 mg, 18.7 μmol) was refluxed with thionyl chloride (300 μl, 4.14 mmol) for 30 min. Excess thionyl chloride was then removed *in vacuo*, and the resultant solid was dissolved in dichloromethane (250 μl). To this solution was added **20** (4.93 mg, 12.5 μmol) and

triethylamine (8.71 µl, 62.5 µmol) in dichloromethane (300 µl) and the reaction was stirred at room temperature for 12 h. After solvent removal *in vacuo*, the resultant residue was purified by flash chromatography (SiO_2, step-wise gradient from 8:1 to 2:1 hexane/acetone) yielding the amide as a white solid (4.70 mg, 7.96 µmol, 64%).



21 **22**

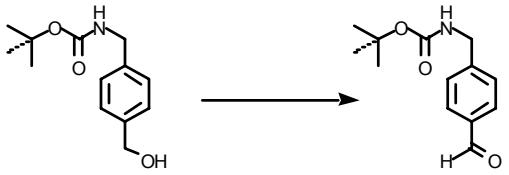
***N*-Methyl-*N*'-(3-Pyridinylbenzyl)-*N*'-(3-Chlorobenzo[b]thiophene-2-carbonyl)-1,4-Diaminocyclohexane (SAG; 22).** Trifluoroacetic acid (300 µl, 3.89 mmol) was added to **21** (2.35 mg, 3.98 µmol) and after complete solvation (1-2 min) the trifluoroacetic acid was removed by a stream of nitrogen gas. The residue was then dissolved in chloroform (1 ml) and washed with saturated aqueous $NaHCO_3$ (2 × 1 ml). The organic layer was isolated, dried over $MgSO_4$, and concentrated *in vacuo* to yield the secondary amine as a white waxy solid (1.97 mg, 4.02 µmol, 100%).



23

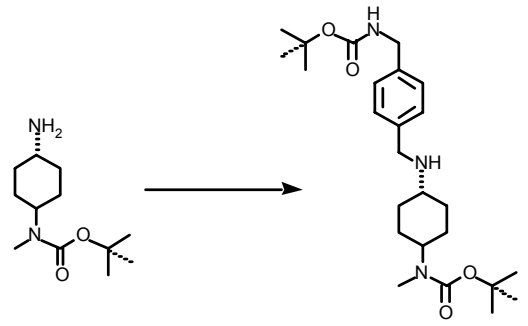
***N*-Boc-4-Hydroxymethylbenzylamine (23).** Lithium aluminum hydride (9.06 ml of a 1 M solution in THF, 9.06 mmol) was added to a solution of 4-cyanobenzaldehyde (250 mg, 1.81 mmol) in THF (10 ml). The reaction was refluxed for 3 h and then quenched with water (5 ml) and aqueous KOH (15 ml of a 10% solution). After extracting the mixture with chloroform (20 ml), the organic layer was dried over $MgSO_4$, and

concentrated *in vacuo*. BOC-ON (342 mg, 1.39 mmol) and triethylamine (387 µl, 2.78 mmol) were then added to a solution of the resultant residue in dichloromethane (5 ml). After the reaction was stirred at room temperature for 6 h, solvent was again removed *in vacuo.* Purification by flash chromatography (SiO_2, step-wise gradient from 8:1 to 2:1 hexane/acetone) yielded the alcohol as a white solid (223 mg, 940 µmol, 52%).



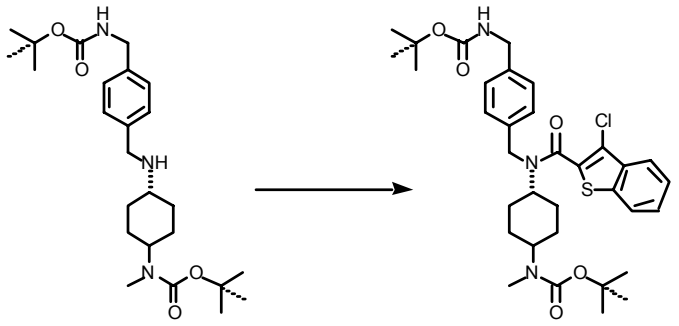
23 **24**

***N*-Boc-4-Aminomethylbenzaldehyde (24).** Dimethylsulfoxide (612 µl, 8.63 mmol) was added to a solution of oxalyl chloride (377 µl, 4.32 mmol) in dichloromethane (5 ml) at –78°C. After the mixture was stirred at –78°C for 10 min, a solution of **23** (205 mg, 864 µmol) in dichloromethane (5 ml) was added, and the reaction was stirred at –78°C for another 30 min. The oxidation was completed by the addition of triethylamine (2.41 ml, 17.3 mmol) to the solution, which was stirred at –78°C for 10 min and then allowed to warm to room temperature. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (10 ml) and the organic layer was isolated. The organic layer was then washed with 1 M HCl (2 × 10 ml) and saturated aqueous $NaHCO_3$ (10 ml), dried over $MgSO_4$, and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, step-wise gradient from 8:1 to 2:1 hexane/acetone) yielded the aldehyde as a white solid (178 mg, 757 µmol, 88%).



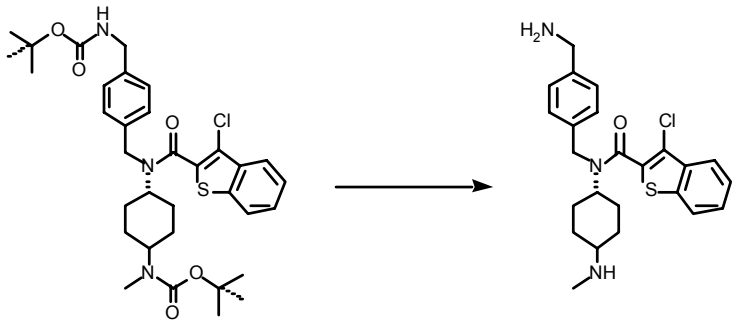
18 **25**

***N*-Boc-*N*-Methyl-*N*'-(*N*''-Boc-4-Aminomethylbenzyl)-1,4-Diaminocyclohexane (25).** A solution of **18** (35.4 mg, 155 μmol) and **24** (54.8 mg, 233 μmol) in ethanol (1.5 ml) was treated with 4 Å molecular sieves and stirred at 70°C for 2 h. The mixture was allowed to cool to room temperature, upon which $NaBH_4$ (15.0 mg, 388 μmol) was added. After the reaction was stirred at room temperature for 5 h, it was diluted with saturated aqueous $NaHCO_3$ (3 ml) and chloroform (4 ml), and the organic and aqueous layers were separated. The aqueous layer was extracted further with chlorofom (2 × 2 ml) and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, step-wise gradient from 10:1 to 5:1 dichloromethane/ethanol) yielded the secondary amine as a brown oil (29.3 mg, 65.5 μmol, 42%).



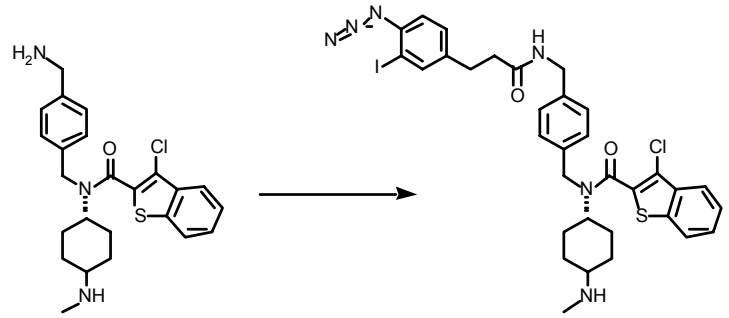
25 **26**

***N*-Boc-*N*-Methyl-*N*'-(*N*"-Boc-4-Aminomethylbenzyl)-*N*'-(3-Chlorobenzo[b] thiophene-2-Carbonyl)-1,4-Diaminocyclohexane (26).** 3-Chlorobenzo[b]thiophene-2-carboxylic acid (14.3 mg, 67.0 μmol) was refluxed with thionyl chloride (600 μl, 8.28 mmol) for 30 min. Excess thionyl chloride was then removed *in vacuo*, and the resultant solid was dissolved in dichloromethane (500 μl). To this solution was added **25** (20.0 mg, 44.7 μmol) and triethylamine (31.2 μl, 224 μmol) in dichloromethane (500 μl), and the reaction was stirred at room temperature for 5 h. After solvent removal *in vacuo*, the resultant residue was purified by flash chromatography (SiO_2, step-wise gradient from 8:1 to 4:1 hexane/acetone) yielding the amide as a white solid (30.6 mg, 47.6 μmol, ~100%).



26 **27**

***N*-Methyl-*N*'-(4-Aminomethylbenzyl)-*N*'-(3-Chlorobenzo[b]thiophene-2-Carbonyl)-1,4-Diaminocyclohexane (27).** Trifluoroacetic acid (400 µl, 5.19 mmol) was added to **26** (10.0 mg, 15.6 µmol) and after complete solvation (1-2 min) the trifluoroacetic acid was removed by a stream of nitrogen gas. The residue was then dissolved in chloroform (1 ml) and washed with saturated aqueous $NaHCO_3$ (2 × 1 ml). The combined aqueous layers were then re-extracted with chloroform (1 ml). The combined organic layers were dried over Na_2SO_4, and concentrated by a stream of nitrogen gas to yield the diamine as a colorless oil (5.48 mg, 12.4 µmol, 80%).



27 **28**

***N*-Methyl-*N*'-(*N*''-Azidoiodophenylpropionyl-4-Aminomethylbenzyl)-*N*'-(3-Chlorobenzo[b]thiophene-2-Carbonyl)-1,4-Diaminocyclohexane (PA-SAG; 28).** Azidoiodophenylpropionyl *N*-hydroxysuccinimide ester (2.00 mg, 4.83 µmol) was added to a solution of **27** (2.13 mg, 4.83 µmol) in chloroform (400 µl). The reaction was stirred at room temperature for 19 h and evaporated to dryness by a stream of nitrogen gas.

Purification by flash chromatography (SiO_2, step-wise gradient from 40:1:0.4 to 10:1:0.1 chloroform/methanol/triethylamine) yielded the azide as a colorless oil (2.90 mg, 3.91 μmol, 81%).

Preparation of ^{125}I-Labeled 28. ^{125}I-labeled azidoiodophenylpropionyl *N*-hydroxysuccinimide ester (0.125 mCi, specific activity = 2200 Ci/mmol, 56.8 pmol; Marty Arbabian and Dr. Arnold Ruoho, Univ. of Wisconsin) in ethyl acetate (~1 ml) was concentrated to a volume of approximately 20 μl by a stream of nitrogen gas. The concentrated solution was diluted with ethyl acetate (25 μl) and mixed with **27** (1.0 mg, 2.26 μmol) in chloroform (50 μl). The reaction mixture was incubated without stirring for 12 h at room temperature and then concentrated to approximately 10 μl by a stream of nitrogen gas. The residue was resuspended in chloroform (200 μl) and purified by flash chromatography (SiO_2, step-wise gradient from 40:1:0.4 to 10:1:0.1 chloroform/methanol/triethylamine) to yield the radiolabeled azide. Fractions containing the desired product were pooled, concentrated by a stream of nitrogen gas, and resuspended in methanol (250 μl). The solution was then reconcentrated by a stream of nitrogen gas, resuspended in methanol (125 μl), and stored at –20°C in the dark.